DECLARATION

Opposition to European Patent No. 120 694
Patentee: Celltech Therapeutics Limited
Opponent: Pharmacia AB

I, Lars Abrahmsén, do hereby declare and state as follows:

1. I am currently employed as a project team leader by Pharmacia & Upjohn in Stockholm, Sweden. I have the qualification of PhD. I have worked on production of proteins in bacteria since 1982 and I am currently working on production of antibodies in *E.coli*. A copy of my curriculum vitae is attached.

2. I have been asked to comment on the question of whether the opposed patent (EP 120 694) and its GB priority document (GB 8308235) contain sufficient information to enable production of functional immunoglobulins or functional immunoglobulin fragments in bacterial cells. I understand that the GB priority document was filed in March 1983 and that the European application for the opposed patent was filed in March 1984.

3. I have read and am familiar with the opposed patent and the priority document and the submissions filed on behalf of Celltech on 6 June 1995 and 8 May 1996.

The disclosures of the priority document and the patent

4. Neither the priority document nor the patent contain Examples showing production of a functional immunoglobulin or a functional immunoglobulin fragment in a single bacterial cell. The Examples in the priority document merely show separate expression of light and heavy chains in separate *E.coli* host cells. The Examples in the patent show production of light and heavy chains in the same *E.coli* host cell, but functional immunoglobulin was not obtained in the cell; rather, functional immunoglobulin was only obtained outside the cell after extraction and reconstitution of the chains (see pages 13-15 of the patent).

5. I believe that the reason that functional immunoglobulin was not obtained in a single bacterial cell is that, although the individual light and heavy chains were produced in the cell, they did not fold and assemble in the cell to give functional immunoglobulin. In order to produce functional immunoglobulin in a single bacterial cell, it is necessary to find a solution to this "folding and assembly" problem. I cannot find any appreciation of the problem in either the priority document or the patent, let alone guidance as to how the problem might be solved.

6. The priority document contains almost no information of any practical use for bacterial production beyond what was already known before the priority date. Amster (D10) shows the production of part of a light chain in a bacterial cell and Kemp (D16) shows production of part of a heavy chain in a bacterial cell, which is similar to what is

shown in the priority document. The fact that Amster and Kemp describe production of only part of light and heavy chains is not important because, in order to produce complete chains, it would simply be necessary to replace the partial DNA sequences described in Amster and Kemp with complete sequences.

Celltech's and Genentech's efforts

7. There is strong support in addition to that in the priority document and the patent for believing that Celltech made a great effort to produce functional immunoglobulin in a single bacterial cell at around the priority date and the European filing date, but failed to do so because they failed to appreciate and to solve the folding and assembly problem. Such support is found in publications of the alleged inventors, namely Boss III (D44), Boss IV (D46) and Wood et al (including Boss) (D48).

8. Boss III (D44) appears to correspond to the experiment described in the patent on pages 13-15. The document describes transformation of heavy and light chain DNA sequences into *E.coli*. However, both proteins were found as insoluble products and functional immunoglobulin was not produced in the cell. Functional immunoglobulin was only obtained after the intracellularly produced insoluble chains had been solubilised and associated outside the cells.

9. In Boss IV (D46), when referring to their own experiments, the alleged inventors stated that:

> "These cloned heavy and light chains were expressed together in the same *E.coli* cells but not as functional antibodies" (page 12, left column, lines 12-10 from the bottom).

10. The alleged inventors went as far as to say in Boss IV (D46) that their

> "results are disappointing and suggest that *E.coli* is unlikely to prove useful for the production of functional antibodies" (page 12, right column, lines 6-8).

11. In Wood (D48), the alleged inventors said the following with reference to their own work and the work of Cabilly et al (abstract):

> "The co-expression of light and heavy chains in *Escherichia coli* has been reported but functional antibodies were not assembled *in vivo*[6,7]. Furthermore, only low-level assembly of these chains was found *in vitro*."

Reference 6 mentioned in this quotation is Boss III (D44) and reference 7 is Cabilly et al (1984) Proc. Natl. Acad. Sci. USA 81 3273-3277 (hereinafter referred to as "the Cabilly reference").

12. The Cabilly reference shows that the alleged inventors were not the only people who failed to produce functional immunoglobulin in a bacterial cell at about the time of

the priority date and filing date. Genentech also failed. The Cabilly reference, whose authors appear to be employees of Genentech and their colleagues, describes a method in which functional immunoglobulin was only obtained after *in vitro* reconstitution of chains produced in *E.coli*. It is clear from the last sentence of the Discussion on page 3277 that Cabilly et al appreciated the desirability of producing functional antibody in a single bacterial cell. Here it is stated:

> "In addition, the expression of pre-heavy chain (or pre-Fd' segment) and pre-light chain in *E.coli*, yeast, and mammalian cells could be pursued to establish conditions for *in vivo* assembly of secreted antibodies."

However, like the alleged inventors, Cabilly et al did not suggest any solution that enabled production of functional antibody in an *E.coli* cell. In the paragraph bridging the left and right columns on page 3276, Cabilly et al stated that:

> "There is, however, no detectable antibody activity in extracts of *E.coli* coproducing substantial levels of IgG H and L chains (Table 1). This may be due to the highly reducing intracellular environment (35,36), which inhibits disulphide-bond formation, and to the accumulation of gene products in insoluble "refractile bodies" in the cell, a phenomenon noted in many cases of exogenous gene expression in *E.coli* (37). It is also possible that *E.coli* is lacking a protein that might be required for *in vivo* formation of IgG (28)."

13. Cabilly et al filed a patent application based on the work described in the Cabilly reference. This application is EP-A-125 023 (Genentech, D60). The Examples in the application confirm that Genentech did not produce functional antibody in a bacterial cell and that such antibody was only obtained after reconstitution of the chains outside the cell.

The first public disclosures of production of functional immunoglobulin fragment in a bacterial cell

14. I believe that the first publications of bacterial production of functional immunoglobulin fragments are Better (D52), Skerra and Plückthun (D54) and possibly Cabilly I (D50). These documents were published more than 5 years after the priority date.

15. The documents contain additional information regarding vector structure and culture conditions that enabled the folding and assembly problem to be solved. Before the documents were published, such information was not available to scientists working in the field and the priority document and patent do not contain such information.

Appendices A and B of Celltech's submissions of 8 May 1996

16. Celltech use Appendix A and Appendix B to support the argument that the priority document and the patent contain sufficient information to enable production of functional immunoglobulin and functional immunoglobulin fragments in a bacterial cell. However, the Appendices contain about 15 pages of text and 8 Figures which are not present in either the priority document or the patent. I would not find credible the suggestion that procedures along the lines set out in the Appendices are taught in the priority document and the patent. The Appendices contain much information regarding vector structure and culture conditions beyond what is taught in the priority document and patent.

17. Incidentally, I do not believe that it is correct for the Patentee to assume that the "fact that the Ig chains secreted by the bacterial cells are humanised chains is irrelevant" (item 1.5 of Appendix A). The humanization of an antibody can for some reason increase its production level in *E.coli* by as much as 100-fold. In Carter et al (1992) Bio/Technology 10 163-167, it was found that (page 165, left column, first paragraph of the Discussion):

> "The humanised variable domains contribute significantly to the high titers since replacement with corresponding domains from the murine parent antibody reduced expression titers by 100-fold under the same fermentation conditions and by 10-fold under conditions optimised for expression of the chimeric fragment (R.K., B.S. and M.C., unpublished data). These dramatic differences in expression titers between humanized and chimeric molecules probably reflect many factors, which may include differences in the relative expression levels of light and heavy chains, kinetics of membrane transport, folding and association of light and heavy chains plus competing light chain dimerization."

The yield of antibody obtained in Appendix A is very low and it is possible that the yield could be reduced even further if the antibody had not been humanized.

Conclusions

18. For the reasons given above, I do not believe that the priority document or the opposed patent contains sufficient information to enable production of functional immunoglobulin or immunoglobulin fragment in a single bacterial cell. It is necessary to find a solution to the folding and assembly problem to achieve such production. The priority document and patent do not appreciate the problem, let alone give guidance as to how the problem could be solved. Information necessary to solve the problem first became available about 5 years after the priority date.

19. In view of the fact that the alleged inventors failed to achieve bacterial production and that they made statements at about the time of the filing date such as "*E.coli* is unlikely to prove useful for the production of functional antibodies" (Boss IV, 1985, D46), I do not find credible the argument by Celltech's Patent Attorney that the alleged inventors' disclosures in the priority document and patent provide sufficient information. I attach much more significance to the statements made by the alleged inventors and Genentech's scientists at about the time of the filing date than to the arguments made now

by Celltech's Patent Attorney over 10 years later.

20. All statements made herein of my own knowledge are true and all statements on information and belief are believed to be true.

This . day of May 1996

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Lars Abrahmsén, PhD

CURRICULUM VITAE

Name: Lars Bertil Abrahmsén

Born: October 6th, 1957, Stockholm, Sweden.

Nationality: Swedish

EDUCATION AND TRAINING

1978-1982 Royal Institute of Technology, Stockholm, Sweden.
Specialized in Chemistry, primarily organic, physical and biochemistry. My graduate thesis was in the latter subject involving the development of a positive selection system for cloning in *Bacillus subtilis.*

1982-1983 Research Associate in Physical chemistry at the University of New Orleans, New Orleans, Louisiana, USA (1982-83). The work involved calculating molecular structures and writing programs in Fortran.

1983-1988 Graduate student in Molecular Biology at the department of Biochemistry and Biotechnology, the Royal Institute of Technology. Thesis: "Different strategies to utilize the staphylococcal protein A gene in the expression of recombinant proteins in bacteria."

1988-1990 Post Doctorial research in Protein Engineering at Genentech Inc., South San Francisco, California, USA in the laboratory of Dr. James A. Wells. The main objective was to turn the *B. subtilis* protease subtilisin into a peptide ligase. I characterized four successful versions of such a peptide ligase and showed in a semisynthesis experiment that these are practically useful.

EMPLOYMENTS

1991- Pharmacia and Upjohn, previously Kabi Pharmacia, Pharmacia BioScience Center, previously KabiGen. As a molecular biologist.

PUBLICATION LIST

<u>Papers in molecular biology.</u>

1) **Nilsson, B., Abrahmsén, L. and M. Uhlén.** Immobilization and purification of enzymes with staphylococcal protein A gene fusion vectors.
EMBO J. 4: 1075-1080 (1985).

2) **Abrahmsén, L., Moks, T., Nilsson, B., Hellman U. and M. Uhlén.** Analysis of the signals for secretion in the staphylococcal protein A gene.
EMBO J. 4: 3901-3906 (1985).

3) **Moks, T., Abrahmsén, L., Nilsson, B., Hellman, U., Sjöquist, J. and M. Uhlén.** Staphylococcal protein A contains five IgG-binding regions. Eur. J. Biochem. 156: 637-643 (1986).

4) **Abrahmsén L., Moks. T., Nilsson, B. and M. Uhlén.** Secretion of heterologous gene products to the culture medium of *Escherichia coli*. Nucl. Acids Res. 14: 7487-7500 (1986).

6) **Uhlén, M., Moks, T., Abrahmsén, L. and B. Nilsson.** Using genetics to facilitate protein purification. In: Fifth Intern. Symp. on the Genetics of Industrial Microorganisms (Eds. M. Alacevic, D. Hranucli, Z. Toman), pp. 205-211, 1986.

7) **Abrahmsén, L., Moks, T., Uhlén, M. and B. Nilsson.** Signals for secretion in the staphylococcal protein A gene - secretion of heterologous gene products to the growth medium of *E.coli*.In: Fifth Intern. Symp. on the Genetics of Industrial Microorganisms (Eds. M. Alacevic, D. Hranueli, Z. Toman), pp. 439-445, 1986.

8) **Nilsson B., Moks. T., Jansson, B., Abrahmsén, L., Elmblad, A., Holmgren, E., Henrichson, C., Jones, T. A. and M. Uhlén.** A syntethic IgG-binding domain based on the staphylococcal protein A. Protein Engineering **1**: 107-113 (1987).

9) **Moks, T., Abrahmsén, L., Holmgren, E., Bilich, M., Olsson, A., Pohl, G., Sterky, C., Hultberg, H., Josephson, S., Holmgren, A., Jörnvall, H., Uhlén, M. and B. Nilsson.** Expression of human insulin-like growth factor I in bacteria: use of optimized gene fusion vectors to facilitate protein purification. Biochemistry **26**: 5244-5250 (1987).

10) **Moks, T., Abrahmsén, L., Österlöf, B., Josephson, S., Östling, M., Enfors, S.-O., Persson, I.-L., Nilsson, B. and M. Uhlén.** Large-scale affinity purification of a human peptide hormone recovered from the culture medium of *Escherichia coli*. Bio/Tcchnology 5: 379-382 (1987).

11) **Uhlén, M., Moks, T., Abrahmsén, L. and B. Nilsson.** Genetic design to facilitate recovery of bioproducts, secretion in *E.coli* and affinity purification. In: Biosensors, Instrumentation and Processing (The World Biotech Report, Vol. 1), pp. 15-23, On Line publications, London, UK, 1987.

12) **Nygren, P.-Å., Eliasson, M., Palmcrantz, E., Abrahmsén, L. and M. Uhlén.** Analysis and use of the serum albumin binding region of streptococcal protein G. J. Mol. Recognition. **1**: 69-74 (1988).

13) **Hellebust, H., Murby, M., Abrahmsén, L., Uhlén, M. and S.-O. Enfors.** Different approaches to stabilize a recombinant fusion protein. Bio/Technology 7: 165-168 (1989).

14) **Uhlén, M. and L. Abrahmsén.** Secretion of recombinant proteins in the culture medium by Escherichia coli and Staphylococcus aureus. Biochem. Soc. Trans. **17**: 340-341 (1989).

15) **Nilsson, B. and L. Abrahmsén.** Fusions to staphylococcal protein A. Methods Enzymol., **185**: 144-161 (1990).

16) **Abrahmsén, L., Tom, J., Burnier, T., Butcher, K. A., Kossiakoff, A. and J. A. Wells.** Engineering subtilisin and its substrates for efficient ligation of peptide bonds in aqueous solution. Biochemistry **30**: 4151-4159 (1991)

17) **Carter, P., Abrahmsén, L. and J. A. Wells.** Probing the mechanism and improving the rate of substrate-assisted catalysis in Subtilisin BPN'. Biochemistry **30**: 6142-6148 (1991).

18) **Dohlsten, M., Abrahmsén, L., Björk, P., Lando, P.A., Hedlund, G., Forsberg, G., Brodin, T., Gascoigne, N.R.J., Förberg, C., Lind, P. and T. Kalland.** Monoclonal antibody-superantigen fusion proteins: tumor-specific agents for T-cell-based tumor therapy. Proc Natl. Acad. Sci. USA **91**:8945-8949 (1994).

19) **Abrahmsén, L., Dohlsten, M., Segrén, S., Björk, P., Jonsson, E. and T. Kalland.** Characterization of two distinct MHC class II binding sites in the superantigen staphylococcal enterotoxin A. EMBO J. **14**:2978-2986 (1995).

20) **Abrahmsén, L.** Superantigen engincering. Curr. Opin. Struct. Biol. 5: (1995).

2) **Politzer, P., Domelsmith, L. N. and L. Abrahmsén.** Electrostatic potentials of strained systems: Cubane, homocubane and bishomocubane. J. Phys. Chem. **88**: 1752-1758 (1984).

3) **Politzer, P., Abrahmsén, L. and P. Sjöberg.** Effects of amino and nitro substituents upon the electrostatic potential of an aromatic ring. J. Amer. Chem. Soc. **106**: 855-860 (1984).

4) **Politzer, P., Laurence, P. R., Abrahmsén, L., Zilles, B. A. and P. Sjöberg.** The aromatic C-NO_2-bond as a site for nucleophilic attack. Chem. Phys. Letters **111**:75-78 (1984).

5) **Owens, F. J., Jayasuraiya, K., Abrahmsén, L. and P. Politzer.** Computational analysis of some properties associated with nitro groups in polynitroaromatic molecules. Chem. Phys. Letters **116**: 434-438 (1985).

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1) **Abrahmsén, L., Moks, T., B. Nilsson and M. Uhlén.** Construction of an IgG binding protein to facilitate downstream processing using protein engineering. SE 8505922 (1985)

2) **Abrahmsén, L., Moks, T., B. Nilsson and M. Uhlén.** A method to export gene products to the growth medium of Gram negative bacteria. SE 8505921 (1985)

3) **Nygren, P.-Å., Abrahmsén, L. and M. Uhlén** Recombinant fusion protein, its use and a recombinant vector. U.S. Pat. Appl. 07/322,726 (1989).

4) **Nygren, P.-Å., Abrahmsén, L. and M. Uhlén** Production of fused proteins or polypeptides. U.S. Pat. Appl. 07/863,539 (1989).

5) **Abrahmsén, L., Burnier, T. and J. A. Wells** Serine protease variants having peptide ligase activity. U.S. Pat. Appl. 07/566,026 (1990).

6) **Abrahmsén, L., Holmgren, E., Lake, M., Lind, P. and B. Nilsson** Expression system for producing apolipoprotein A1-M. U.S. Pat. Appl. 07/305,650 (1992).

7) **Abrahmsén, L., Björk, P., Dohlsten, M. and T. Kalland.** A conjugate between a modified superantigen and a target-seeking compound and the use of the conjugate. SE 9402430 (1994)